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Research Article

Pathogenic Insights into *Toxoplasma gondii*: Host Interaction, Immune Evasion, and Therapeutic Strategies

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Abstract

Toxoplasma gondii is a globally distributed, obligate intracellular parasite that infects virtually all warm-blooded animals, including humans. Although infection is typically asymptomatic in immunocompetent individuals, it can lead to severe disease in immunocompromised patients and fetuses during congenital transmission. The parasite's ability to persist lifelong within host tissues, particularly in immune-privileged sites such as the brain and eyes, underscores its complex strategies for host manipulation and immune evasion.

This review focuses on recent advances in the understanding of *T. gondii* pathogenesis, with a particular emphasis on host–parasite interactions, molecular mechanisms of immune evasion, and emerging therapeutic approaches. We explore the role of key effector proteins-especially rhoptry (ROPs) and dense granule (GRAs)-in altering host immune signaling, suppressing inflammatory responses, and promoting intracellular survival. Recent findings on mitochondrial hijacking through host mitochondrial association (HMA) and modulation of host non-coding RNAs further reveal the parasite's sophisticated manipulation of host cell biology.

Chronic toxoplasmosis remains a major therapeutic challenge, as existing treatments such as pyrimethamine-sulfadiazine are effective only against the actively replicating tachyzoite stage and have significant toxicity. This review discusses novel treatment strategies including apicoplast-targeting agents, host-directed therapies, nanoparticle drug delivery systems, and CRISPR-Cas9-based functional genomic approaches to identify essential parasite genes and new drug targets.

This review aims to provide a comprehensive and current perspective on *T. gondii's* biology and pathogenesis. The review also highlights gaps in current knowledge, such as the limited understanding of bradyzoite persistence mechanisms and ineffective cyst-targeted therapies. By narrowing our focus to the mechanistic and therapeutic aspects of *T. gondii*, we aim to inform future research and guide the development of more effective interventions against both acute and chronic toxoplasmosis.

Keywords: *Toxoplasma gondii*; Pathogenesis; Diagnosis; Treatment; Transmission; Vaccine Development

Introduction

Toxoplasma gondii is a highly successful protozoan parasite that infects virtually all warm-blooded animals, including humans. Belonging to the phylum Apicomplexa, this obligate intracellular organism is the causative agent of toxoplasmosis, a disease with a broad clinical spectrum ranging from asymptomatic infection in immunocompetent individuals to severe neurological or ocular disease, particularly in immunocompromised patients and congenitally infected infants. Globally, approximately one-third of the human population is estimated to carry latent *T. gondii* infection, with regional variation influenced by dietary habits, sanitation, and climate. Despite this high prevalence and public health significance, effective therapeutic options remain limited, especially for chronic and cyst-forming stages of the parasite [1].

The parasite has a complex life cycle involving both sexual and asexual reproduction. Sexual reproduction is confined to the intestines of felids, the definitive hosts, where the parasite undergoes gametogenesis and sheds oocysts. Asexual reproduction occurs in the intermediate hosts, which includes humans, by replicating rapidly as tachyzoites or slowly reproducing as bradyzoites encysted within tissue cysts. Infections are often transmitted via ingestion of food or water contaminated with sporulated oocysts or consumption of undercooked meat containing tissue cysts. Transplacental transfer may occur from a mother to her fetus infected with T. gondii, particularly as the primary infection occurs during pregnancy. Following ingestion, tachyzoites disseminate throughout the body, invade multiple nucleated cells, and cause acute infection. The innate immune response may eventually force the parasite to reproduce as the latent bradyzoite stage, forming tissue cysts (particularly in the brain, eyes, and muscles) for the remainder of the host's life [2].

In the field of parasitology and immunology, elucidating the mechanisms by which T. gondii establishes an infection, evades immune detection, and persists in host tissues, has been a key area of study. In recent years, tremendous progress has been made in dissecting the complex interplay between T. gondii and its host, including the identification of virulence factors/secreted proteins from the parasite (e.g., the rhoptry (ROP) and dense granule (GRA) proteins), released during and following the initial invasion into a host cell, that modulate host cell signaling, gene expression, and immune surveillance, thus facilitating the parasite's ability to replicate and survive in the host. For example, ROP16 activates host STAT3 and STAT6 to reduce the expression of proinflammatory cytokines, while ROP18 inactivates immunity-related GTPases (IRGs), thus protecting the parasitophorous vacuole (PV) from destruction by the host [3].

In addition to effector-mediated modulation, T. gondii also alters host cell architecture and function at the organelle level. For example, the parasite recruits host mitochondria to the PV membrane (walled off site of infection) through proteins such as MAF1b through a process referred to as association of host mitochondrial association (HMA) . This interaction may help the parasite adapt to and gather nutrients from its host, while also potentially distracting the host's innate immune signaling. Moreover, T. gondii infection modifies host non-coding RNA expression, specifically microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) that are increasingly being thought of as regulators of immune responses. Changes in the normal regulatory functions of miR-155 and miR-146a can directly influence the host's capacity to mount a proper proinflammatory response emphasizing that T. gondii can hijack the host's epigenetic strategies [4].

These sophisticated evasion strategies allow *T. gondii* not only to survive but to establish chronic infections, particularly in immune-privileged sites such as the brain. In immunocompromised individuals, such as those with HIV/AIDS or organ transplant recipients, reactivation of latent infection can lead to life-threatening toxoplasmic encephalitis. Recent studies have revealed that bradyzoites within tissue cysts suppress host antigen presentation pathways and evade microglial surveillance, contributing to persistent infection with minimal immune activation. Despite extensive research, no existing therapy can effectively eliminate bradyzoite cysts, highlighting a critical gap in toxoplasmosis management [5].

Therapeutic strategies remain largely limited to combinations of pyrimethamine, sulfadiazine, and leucovorin, which are effective against tachyzoites but not cysts. These regimens are associated with considerable toxicity and poor patient compliance. Consequently, there is growing interest in developing novel treat-

ments, including host-directed therapies, apicoplast-targeting compounds, and nanoparticle drug delivery systems. In parallel, genome-wide CRISPR-Cas9-based screens have facilitated the identification of essential parasite genes involved in invasion, replication, and immune evasion, opening new avenues for targeted drug discovery [6].

Given the expanding body of research and the ongoing clinical challenges posed by toxoplasmosis, a comprehensive and timely synthesis of current knowledge is essential. This review focuses specifically on the molecular and cellular mechanisms that enable *T. gondii* to invade host cells, evade immune responses, and establish chronic infection. Special emphasis is placed on recent discoveries, including effector protein functions, host cell remodeling, mitochondrial interactions, immune modulation, and emerging therapeutic targets. By narrowing the scope to these fundamental aspects of parasite-host interplay, this review aims to provide meaningful insights into the biology of *T. gondii* and identify promising directions for future therapeutic interventions [5,6].

Epidemiology of *Toxoplasma gondii*

Toxoplasma gondii is a protozoan parasite that, although distributed worldwide, has the capacity to infect any warm-blooded animal, including humans. Because of the complexity of environmental, cultural, and socioeconomic factors involved, the seroprevalence rates of *T. gondii* are highly variable throughout the world. Knowledge of such regional disparities forms the basis for devising health interventions using a public health approach, supported by improved global management of toxoplasmosis.

Global seroprevalence and influencing factors

Based on the presence of antibodies against the parasite, an estimated one-third of all humans have been exposed to *T. gondii*. However, there is significant heterogeneity within such rates because of variation in socioeconomic position of the investigated communities, food preferences, public health conditions, and environment. In Europe, the rate of seroprevalence ranges from 20% to 60%. Higher rates are observed in countries such as France and Germany, where the dietary practices involve the consumption of undercooked or raw meat, thereby increasing the risk of infection with *T. gondii*. The moderate climate in a large part of Europe is also favorable for the survival of *T. gondii* oocysts in the environment. Seroprevalence is usually low, ranging from 10% to 30%, in the United States and Canada. Such low prevalence rates are related to good public health infrastructure, easily accessed treated water supplies, and strong cultural traditions that promote well-cooked meat. In addition, the cold climate in some areas helps limit the environmental survival of the oocysts [7]. Some of the highest seroprevalence rates are found in Latin America, especially in Brazil, where the seroprevalence can be higher than 60%. Contributing factors include warm and humid climates, which allow oocysts to survive for extended periods outside a host; high levels of cat ownership; and dietary habits involving the consumption of undercooked meat. In these areas, socio-economic disparities also make a difference, with lower-income populations at higher exposure risks because of poor sanitation and lack of health education. Seroprevalence rates are generally high in Africa, mostly over 50% though rates can be quite variable depending upon the region. High rates occur across diverse environmental conditions and cultural practices; in many cases, people consume home-raised or uninspected meat. Poor water treatment and sanitation in some areas further contribute to the transmission of *T. gondii*. Even within Asia, seroprevalence rates range from 5 to 30%, depending on the region and other related risk factors like food and cat ownership. In sharp contrast, however, the seroprevalence of other countries within the same geographical region reveals overwhelmingly low rates, in part due to effective public health intervention and diet that avoids parasite exposure; examples include South Korea and Japan [8].

Epidemiology of *Toxoplasma gondii* in India

The peculiar epidemiological profile of *T. gondii* in the country with its geographical diversity in climatic conditions, cultural practices, and socio-economic disparities has been noted. Seroprevalence of *T. gondii* varies between 20% and 45% in India; such variations are wide within different regions and population groups. Seroprevalence rates as high as 20% to 30% have also been observed in Northern India. Among the causes that may account for lower infection rates are cultural dietary habits such as vegetarianism or ingesting only well-cooked foods. In some places, the climate is relatively cool and may limit the oocysts' ability to survive in the environment for longer periods of time. This contrasts with Southern India, which has a warm humid climate and a much higher

seroprevalence, usually around 30%. A more favorable environment for oocyst survival and specific cultural practices, including consumption of undercooked meat, may help explain these higher rates. There is a significantly higher seroprevalence between urban and rural settings in India. Rural settings are typically higher because of increased contact with animals including domestic or stray cats and consumption of untreated water. All these elements paired with limited access to healthcare and sanitation, ultimately increase the risk of T. gondii transmission for rural populations [9].

Transmission dynamics and public health implications

T. gondii is transmitted by well-recognized routes of infection, namely, the ingestion of oocysts from contaminated environments, undercooked meat containing tissue cysts, and vertical transmission from mother to fetus. Infection rates can vary greatly between regions and populations and depend upon demographic, environmental, and cultural influences.

- **Dietary Habits**: Dietary practice indeed is one important factor in the dynamics of *T. gondii* transmission. A major risk factor is the consumption of raw or undercooked meat, particularly pork, lamb, and game. Indeed, regions with relatively high seroprevalence rates are parts of Europe and Latin America, whose cultural practices include such dietary habits [10].
- **Cat Ownership and Environmental Contamination**: As cats are the definitive hosts of *T. gondii*, they shed oocysts in their feces, which may easily contaminate soil, water, and food. In populations with high densities of cat ownership and those frequently exposed to such contaminated environments-such as farmers and gardeners-the risk of infection is higher [11].
- **Waterborne Transmission**: Water supplies have been identified as a source of outbreaks of toxoplasmosis when proper infrastructure for water treatment is lacking. This mode of infection is of serious concern in any country that has limited access to clean water, because a single infected water supply could potentially expose thousands of individuals [10-12].
- **Vertical Transmission**: Infection with *T. gondii* acquired for the first time during pregnancy may result in vertical transmission of the parasite to the fetus, leading to congenital toxoplasmosis. Risk of vertical transmission and severity of the disease depends on the timing of the infection: the first trimester is considered the most critical period [10].
- **Blood Transfusion and Organ Transplantation:** *T. gondii* may be conveyed in the cases of blood transfusions and organ transplantation through an infected donor to a recipient. That is a route of special concern in the cases of immunocompromised patients, like the ones undergoing an organ transplant, because the effect of Toxoplasma infection can be very serious [10].
- **Public Health Interventions**: Education on the safe handling of food, proper cooking of meat, and ways to reduce environmental contamination are efficient public health measures that can markedly reduce the burden of toxoplasmosis. Countries with effective public health campaigns have shown decreased seroprevalence, especially among pregnant women and other at-risk groups [11,12].

Life Cycle of *Toxoplasma gondii*

Toxoplasma gondii has a life cycle that is both sexually and asexually reproducible. The different stages in its life cycle occur in definitive hosts-felids-and intermediate hosts, which also include man and other warm-blooded animals.

Sexual reproduction in cats

The sexual reproduction phase of Toxoplasma gondii occurs only in felids (domestic and wild), which are the definitive hosts for the parasite. This stage occurs in the intestinal epithelium after the cat eats infected prey (e.g., rodents or birds) that has tissue cysts containing bradyzoites. Once ingested, the bradyzoites release in the cat's small intestine and enter epithelial cells to initiate the sexual cycle. In the intestinal epithelium, the bradyzoites then

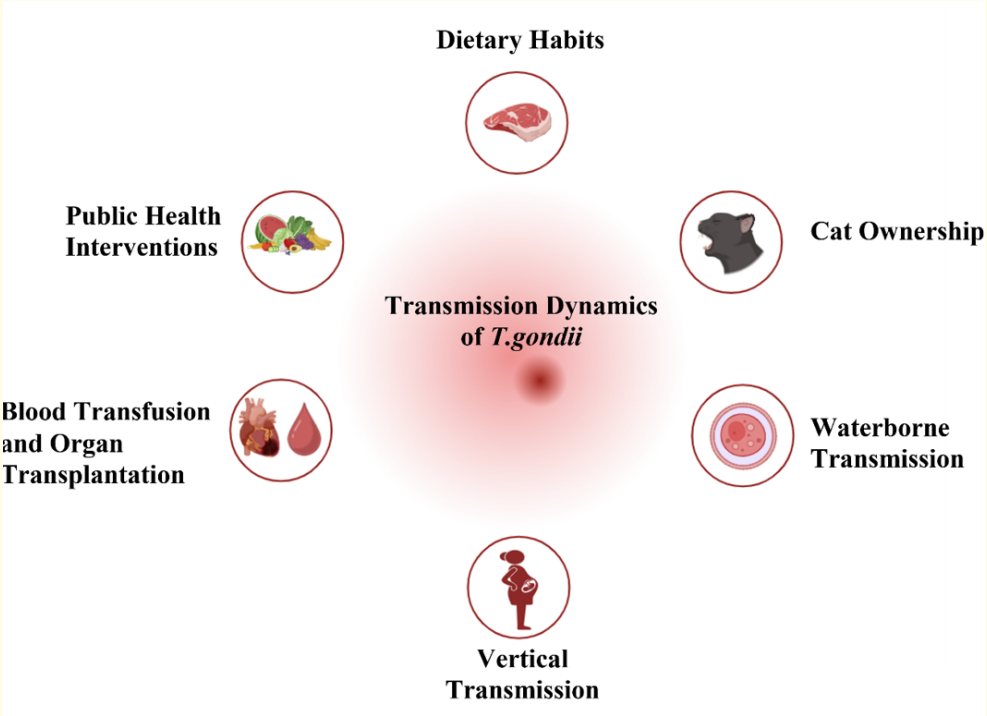

Figure 1: The Key Factors involve in the Transmission Dynamics of *Toxoplasma gondii*.

undergo numerous rounds of asexual replication, before differentiating into gametocytes, male (microgametes) and female (macrogametes). Fertilization happens when microgametes fertilize macrogametes, forming zygotes, which develop into oocysts. Unsporulated oocysts are excreted in cat feces within 3-10 days after the initial infection. A cat that is infected can produce millions of oocysts during this short time and contribute to the environmental contamination [13].

In the environment, the oocysts undergo sporulation within 1–5 days depending on temperature and humidity. Each oocyst is capable of sporulating into two sporocysts, each containing four sporozoites for a total of eight sporozoites per oocyst to become infectious. The infectious forms are incredibly resistant and can survive in soil or water for months and are an important transmission route for humans and other animals. This stage has a vital function in the ecological transmission of T. gondii and fulfills a role in transferring the parasite from one host to another. Sexual recombination occurs only at this stage in the life cycle and leads to genetic variability in parasite strains. Genetic diversity can affect virulence, host susceptibility, and resistance to immune responses.

Therefore, the sexual stage in felids not only is critical for parasite transmission but establishes the overall parasite's ability to adapt and evolve. As a prevention strategy, control of this life cycle step, by removing unrestricted raw meat in a feline diet and limiting access to stray cats, can greatly reduce the environmental burden of infectious oocysts, and therefore lessens the spread of toxoplasmosis, especially in endemic areas [14].

Asexual reproduction in intermediate hosts

In the intermediate hosts, such as humans, domestic animals, rodents, and birds, *T. gondii* is able to reproduce asexually, allowing the parasite to multiply and persist in the intermediate host without having to return to the definitive host. The life cycle begins when the intermediate host ingests sporulated oocysts from food, water, or soil contaminants. Once in the intestines of the host, the oocysts will release sporozoites. The sporozoites will penetrate the intestinal lining and invade host cells.

Once the sporozoites have entered host cells, they will differentiate to tachyzoites, which are the rapidly dividing form of the organism and represent the acute phase of infection. Tachyzoites are able to multiply by the process of endodyogeny, which is characterized by two daughter cells being formed within the mother cell which eventually leads to rupture of the host cell. The rupture of the host cell will allow tachyzoites to disseminate throughout the host through the bloodstream or lymphatic system, where they can infect various tissues, with preference for the brain, the retina, cardiac tissue, and skeletal muscle [15].

The host's immune response triggers a cessation of rapid tachyzoite replication and stimulates tachyzoites to re-develop bradyzoites -- slow replicating "cyst formers" which will encyst in host tissues. These tissue cysts are generally located in immune privileged locations such as the central nervous system (CNS) and muscle tissues. This encapsulating event is the transition from acute to chronic disease, and cysts can be viable for the life of the host. This asexual cycle in intermediate hosts is vital for maintaining T. gondii within the ecosystem and food web. Animals hosting tissue cysts are susceptible to infection by other predators e.g., felids. Humans can be infected by consuming raw or undercooked meat from infected animals or through ingestion of ectocysts. If a woman develops a primary infection during pregnancy, vertically transmitted decomposed cyst can lead to congenital toxoplasmosis [16].

The asexual reproductive role allows *T. gondii* to infect many warm-blooded hosts, but the same activity ensures the species survival and potential transfer through intermediate hosts as felids continue to eat. Insights of the asexual reproductive stage will shape food safety, meat inspection, and public health interventions aimed at prevention of infection and chronic disease management.

Persistence and reactivation

One of the most striking features of *Toxoplasma gondii*'s biology is its ability to stay latent in host tissues by creating tissue cysts containing bradyzoites. Most of these tissue cysts are found in long-lived cells such as neurons and muscle cells, allowing the bradyzoites to persist in a relatively immune-privileged environment. The cyst wall is thick and protects the bradyzoites, allowing those resident cysts to evade immune surveillance and persist for years, if not longer, than the host.

Under functional immunity, tissue cysts remain quiescent and maintain a latent phase without symptoms of acute disease. In instances of impaired immunity (e.g., HIV/AIDS, chemotherapy patients, organ transplant patients), the latent cysts can reactivate and bradyzoites are released from the cysts and differentiate back into tachyzoites which replicate quickly, leading to active infection [17].

Reactivation in the human or other warm-blooded animal host can lead to serious clinical consequences. The most well-known consequence of reactivation is toxoplasmic encephalitis, a potentially life-threatening condition characterized by inflammatory changes and necrosis of the tissues of the brain. Other serious negative outcomes may arise: ocular toxoplasmosis, myocarditis, or, depending upon which tissues contain the parasites in cysts, multi-organ involvement. Persistency and reactivation introduces major challenges to both treatment and management of toxoplasmosis. Currently available antiparasitic treatments are often effective against tachyzoites, and not bradyzoites or the cysts. This leads to reactivation being a significant risk in immunosuppressed patients. Prevention, such as lifelong prophylactic therapies for at-risk patients and careful screening of donors for organs and blood products, is paramount in preventing complications due to the reactivated infection [18].

Additionally, the persistence of the parasite plays an important role in transmission cycles. For example, animals that harbor tolerated dormant/almost dormant cysts 'recover' from Toxoplasma and therefore can serve as a means of transmission if they are consumed undercooked in meat form. As a result of tolerating the cysts, Toxoplasma is also capable of causing zoonotic transmission and facilitate Toxoplasma's amelioration through species in a metabolic lifecyle- not to mention several species can serve as a final host. Ultimately, *T. gondii*'s reservoir capability and reactivation potential allow for a long-term repository in host populations, and serious clinical and epidemiological implications. The molecular aspects of these processes are still being actively researched in toxoplasmosis, with the end goal of eliminating chronic infections and blocking reactivation in vulnerable populations [19].

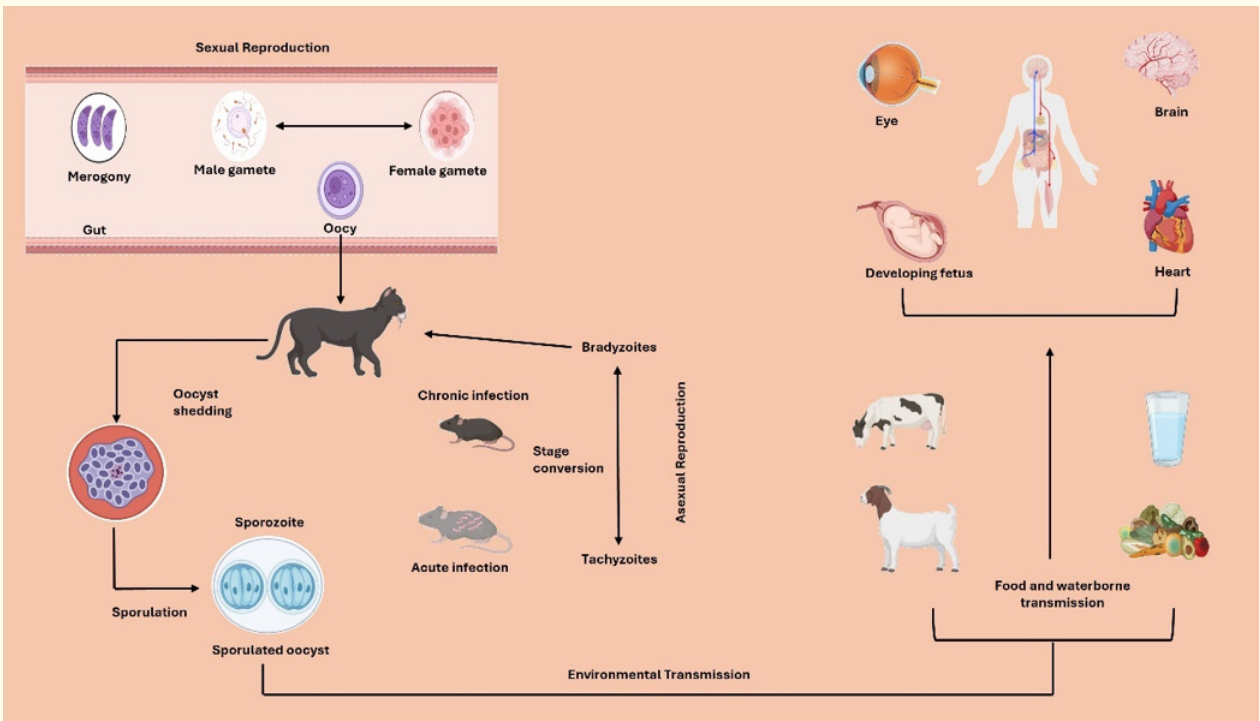

Figure 2: Life cycle of *Toxoplasma gondii* showing sexual reproduction in the feline gut, environmental transmission through sporulated oocysts, asexual reproduction in an intermediate host, and subsequent acute or chronic infection. Potential human routes of infection and target organs for infection are indicated.

Morphology of *toxoplasma gondii*

T. gondii is a protozoan parasite of the phylum Apicomplexa; it is an obligate intracellular organism. The unique morphologies of the parasite relate to their ability to infect a wide array of host organisms and cause disease. The parasites undergo biological changes in successive life cycle events for the tachyzoites, bradyzoites, and sporozoites. These forms morphologically adapt to infect, persist, and proliferate within the tissues of host organisms.

Tachyzoite form

The tachyzoite of Toxoplasma gondii is the quickly replicating and invasive Toxoplasma gondii life-stage responsible for the acute phase of infection. Morphologically, tachyzoites are typically crescent-arc shaped, about 4–7 µm long by 2–3 µm wide, with one pointed end (apical) and one rounded end. The apical end also includes a complex cluster of highly specialized organelles called the apical complex as it contains rhoptries, micronemes, and the conoid. These structures are critical for the parasite's entry into host cells.

When invading the host cells, the conoid, a spiraled microtubule structure that extends from the apical region, helps drive through the host cell membrane. Rhoptries are large, club-shaped organelles that release protein into the PV to form the parasitophorous vacuole (PV) inside the host cell. The vacuole provides a sub-compartment for the parasite to persist and replicate without being destroyed by host immune responses. Micronemes are a smaller and elongated organelle that contain the adhesin proteins released at the time of entry which allow for a very tight attachment of the parasite to promote entry [20].

Another important component is the apicoplast, a plastid-like organelle, of prokaryotic origin. Although it is not engaged in photosynthesis, it is necessary for the synthesis of fatty acids, isoprenoids, and other metabolites. Virtually all metabolic pathways of the apicoplast refer to biosynthetic pathways which are distinctly different from those in human cells, so the apicoplast represents a very attractive target for anti-parasitic drugs. Also, dense granules in the cytoplasm release proteins that change the PV membrane to help the parasite evade host immune mechanisms, and to enhance nutrient acquisition and survival in the intracellular environment.

The tachyzoite's capacity to invade almost all nucleated cell types of virtually all warm-blooded hosts combined with its rapid replication allows for the parasite to spread almost instantly throughout the host. This poses a significant threat to immunocompromised people, such as those with AIDS, and those that have received organ transplants, because the proliferation and survival of tachyzoites, if uncontrolled, can lead to crises including encephalitis. The morphology and intracellular lifestyle of the tachyzoite are overall paramount to the first establishing of the infection; and, the unique organelles and structures, whether dispensable or not, may suggest a way to develop targeted therapies for blocking either parasite entry or survival, or replication, in host cells [21].

Bradyzoite and tissue cyst forms

Toxoplasma gondii has a form known as bradyzoites that is an inactive stage, associated with chronic infection. Bradyzoites are similar in structure to tachyzoites; the only differences are the rate of replication and the ability to form tissue cysts and they are long-lived structures that allow for the persistence of the parasite in host tissues for life. Bradyzoites typically form cysts in neural (brain) and muscular tissues (skeletal and cardiac).

Bradyzoites withing cysts are protected by a cyst wall that shields the bradyzoite from the host immune response and prevents immune effectors from reaching the bradyzoite as a cyst wall is made of concentric layers that are selectively permeable. The cyst wall provides a physical barrier to immune detection while allowing nutrients to pass through. Cysts range in diameter from 10 to 200 µm and contain hundreds to thousands of bradyzoites within each cyst [22].

Bradyzoites replicate slowly compared to tachyzoites, which enables them to survive under an immune response; this slow growth rate also means that bradyzoites are less detectable and will be targeted less readily by antiparasitic therapies since most therapies are ineffective against this stage. Cysts can also stay in a dormant state for a number of years, years or possibly for the life of the host without any symptoms. However, for immunocompromised patients - those with HIV/AIDS, patients with cancer, and patients receiving immunosuppressive drugs - cysts can reactivate with bradyzoites converting back to rapidly replicating tachyzoites. Reactivation can occur leading to toxoplasmic encephalitis, a potentially fatal condition resulting in inflammation of the brain. The ability of the bradyzoites to form resilient tissue cysts allows the parasite to persist in the host for a long time and continue the transmission process, particularly in instances when humans consume undercooked meat from infected animals. It is this bradyzoite form that is responsible for promoting chronic disease and completing T. gondii zoonotic transmission [23].

Due to its role in persistence and reactivation, the bradyzoite phase has been identified as a target for new drugs and vaccine development, as there is potential for new treatments that target the bradyzoite stage to also eliminate reactivation phases of the host in individuals with chronic infections. Understanding the molecular biology of cyst formation, maintenance, and reactivation is necessary for future strategies to eliminate reactivation of chronic disease, and particularly important for vulnerable patients.

Sporozoite form

The infective stage for Toxoplasma gondii is the sporozoite, which is found within oocysts (eggs) that cats shed in their feces. Each oocyst contains two sporocysts, and each sporocyst has four sporozoites, for a total of eight sporozoites per oocyst. Sporozoites

are infectious forms that are important in transmission in the environment and often are the cause of food- or water-borne diseases in humans and other intermediate hosts. Structurally, sporozoites are very similar to tachyzoites, having an apical complex made up of rhoptries, micronemes, and a conoid, all important organelles for invasion of the host cells. When contaminated food or water is ingested, the oocyst wall is digested in the gastrointestinal tract, freeing sporozoites to invade intestinal epithelial cells liver and lymphatics, and to start the asexual replication cycle. They may then differentiate into tachyzoites and spread systemically [24].

A special and defining characteristic of sporozoites is the durability of the oocyst wall. This outer layer is extremely resilient to environmental extremes (heat, cold and chemicals) as it allows the oocysts to survive over a degree of months in soil or water. This environmental endurance plays an important role in the global dissemination of T. gondii and increases the likelihood of new hosts becoming infected by accidental ingestion. Sporozoites are uniquely crucial to environmental survival and transmission of T. gondii, especially in areas that lack sanitation and have high numbers of feral or outdoor cats. For example, consumption of contaminated drinking water has been associated with two outbreaks of toxoplasmosis in humans, including severe illness in immunocompromised patients and pregnant women [25].

Sporozoites are held indoors via the oocyst wall. Due to the significant public health implications of this parasite, it is essential to understand this aspect of sporozoite biology in order to inform and develop preventive measures. The public health umbrella contains many interventions that have been shown to reduce the likelihood of infection such as; improving food safety practices, upgrading public water treatment processes, and managing cat populations. Furthermore, discovering sporozoite specific features such as activation processes or wall degrading enzymes may provide a new avenue of interventions to prevent a first infection [26].

Cytoskeletal and membrane structures

The cytoskeletal and membrane organization of Toxoplasma gondii is essential in keeping its shape and its ability to invade host cells and replicate. One of the most important features of T. gondii is the Inner Membrane Complex (IMC) which is located just under the plasma membrane. The IMC is formed from flattened vesicles which are stabilized and provide structural support with a network of microfilaments and microtubules, allowing for motility and integrity.

The microtubules and microfilaments allow the parasite to exhibit gliding motility (which is one of the most important forms of motility used to locate and invade host cells). The IMC is also important for cell division, particularly the unique process of endodyogeny, a process of asexual reproduction where two daughter cells are formed within an intact mother cell which later bud out from the mother cell. This process allows the parasite to replicate efficiently while keeping internal organization. In T. gondii, the mitochondrion appears elongated and is tightly associated with the IMC. The mitochondrion is used to generate ATP for energy-requiring processes like invasion and replication. The parasite has only one mitochondrion which is efficient for cellular processes, but poses a potentially attractive chemotherapeutic target [27].

In the case of T. gondii, the Golgi apparatus displays functions similar to those in other eukaryotes: it traffics and processes protein. Those proteins are often destined for transport by secreting them into host cells and developing the surface of the parasite for host contact. Thus, the trafficking of secreted proteins is essential for establishing parasitophorous vacuoles, remodeling host cell machinery, and their immune response. In addition, organelles that secrete dense granules, micronemes and roptriies are strongly anchored to the cytoskeleton, which supports their timely secretion of virulence factors. The cytoskeletal system is important to transport the organelles to the apical-end of the parasite, to successfully invade its host cell. In summary, T. gondii and its cytoskeleton/membranes have adopted and compartmentalized the functions from the host and somehow have incorporated them into their well-defined life cycle. The cytoskeletal system and membranes remain vital for producing the cellular morphology of T. gondii, and also motility, invasion, replication and persistence. If any one of the above systems were to diminish their ability to perpetuate, they are at a high risk. For this reason, designs that focus on the cytoskeletal system or membranes of T. gondii, are good candidates for therapeutic testing [28].

Implications for pathogenicity

The morphological characteristics of Toxoplasma gondii are directly related to its potential to cause disease, which influences its ability to invade, replicate, and cause disease in hosts. The parasite's capacity for invading almost any nucleated cell is mostly related to the apical complex which is responsible for attaching to and penetrating cells and forming the parasitophorous vacuole (PV) where it escapes detection by the host's immune system and divides unhindered.

Each of the life cycle stages affects pathogenicity in its own unique way. Tachyzoites are capable of rapid replication, allowing for acute dissemination and crossing biological barriers, including the placenta and blood-brain barrier. This ability allows for serious outcomes in congenital infections and encephalitis in those who are immunocompromised [29].

Bradyzoites allow for infection to persist indefinitely by forming tissue cysts in "immune privileged" areas of the host that include muscle and in particular the brain. Tissue cysts can evade immune clearance and lie in wait allowing for reactivation during any episode of immunosuppression, relapsing the disease. The ability of T. gondii to allow infections to persist indefinitely makes treatment difficult, even permitting the risk of reactivation years or decades after first exposure.

Sporozoites, which are contained in enveloping oocysts that provide environmental exposure and a grave public health threat, will persist for long periods of time in water, soil, and food. Since T. gondii will typically be able to survive for months, the dangerous spread of T. gondii will be very difficult to mitigate unless public health orders are enforced.

Structural components such as the apicoplast, cytoskeleton, and mitochondrion all play crucial roles to maintain the parasite's life cycle and replication. The organelle components are distinct from that of the host and offer some basis for therapeutic discovery. For example, inhibition of apicoplasts could inhibit lipid metabolism that the parasite requires for growth. Other organelles or the IMC and the conoid could be potential therapeutic targets for invading the host cell. It will be important to understand T. gondii's relationships between lifestyle and pathogenicity in order to identify therapeutic strategies. Development of drug strategies based on morphology could develop drug strategies against organelles that are unique to the parasite. Hence, drug strategies to reduce acute infection, and probably cyst formation, or draining chronic reservoirs, can come from morphology [30].

Thus, the structural and morphological attributes of *T. gondii* are not mere biological features but essential tools of pathogenesis, enabling it to be one of the most widespread and resilient parasites infecting humans today.

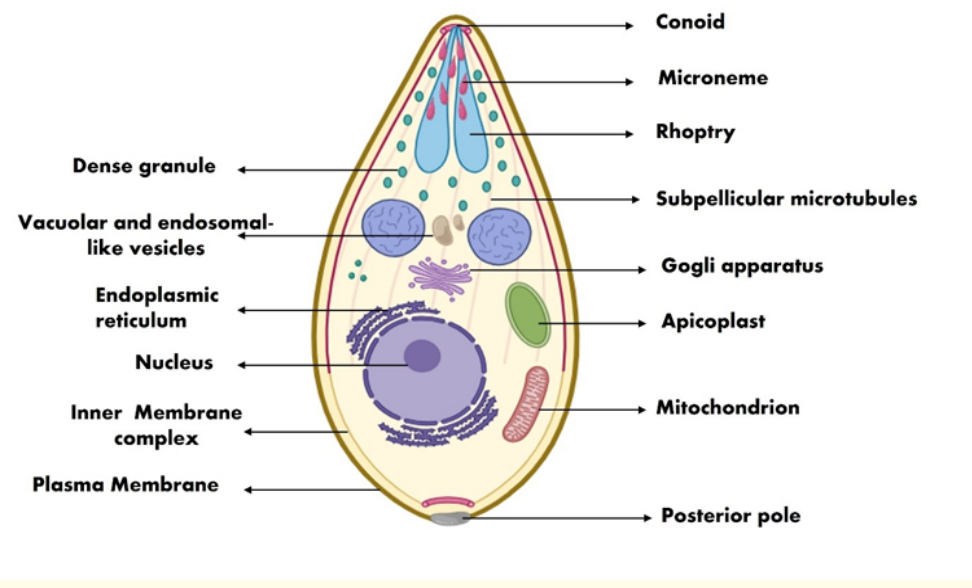

Figure 3: Schematic representation of the ultrastructure of *Toxoplasma gondii*, highlighting key organelles and structures.

Invasion mechanisms of *toxoplasma gondii*

Toxoplasma gondii, a highly adaptable intracellular protozoan parasite, owes much of its success as a globally prevalent pathogen to its extraordinary ability to actively invade and survive within a wide range of nucleated host cells. Unlike many intracellular pathogens that rely on host-driven phagocytosis, T. gondii initiates its own entry through a sophisticated and parasite-driven invasion mechanism. This allows it to bypass traditional immune detection and destruction mechanisms, laying the foundation for both acute and chronic infection.

Host cell recognition and adhesion

The invasion process starts with the initial attachment and recognition of the host cell surface by the parasite. Parasite-derived surface antigens, particularly, SAG1, SAG2 and microneme proteins (MICs) mediate this process. Ligand-receptor interactions take place between parasite surface antigens, which serve as ligands, and specific receptors on the host cell membrane that include glycoproteins and other membrane-associated moieties. This role is functionally selective and not random as the parasite preferentially attaches to its chosen host.

The specificity and strength of this initial adhesion is important for the orientation of the parasite for entry. The parasite will then glide along the surface of the host cell using gliding motility, a unique form of movement that is powered by an internal actin-myosin motor complex just beneath the parasite's plasma membrane. Gliding motility should ensure the parasite maintains contact with the membrane of the host cell and opens up the next step in the process: formation of the entry point [31].

Apical reorientation and conoid insertion

Once the parasite attaches itself to the host cell, T. gondii reorients itself so that its apical complex (the anterior specialized structure) structures is closest to the membrane of the host cell. The apical complex contains many important organelles such as microneme, rhoptries, and a conoidal structure.

Once activated, the conoid extends outside of the parasite cytoskeleton to mechanically breach the membrane of the host. This is aided by the sequential secretion of microneme proteins that serve two functions: first, adhesion signalling, and second, signal transduction events in the host cell to prime it for invasion (presumably to facilitate a less energetic penetration) [32].

Formation of the Moving Junction (MJ)

The next critical step is the creation of a moving junction (MJ)-a narrow, ring-like structure formed between the parasite and host membrane. The junction is created via a synchronized efflux of rhoptry neck proteins (RONs) from the parasite into the host cell including RON2, RON4, and RON5, that become incorporated into the host membrane. At the same time, AMA1, a transmembrane protein from the parasites micronemes, electronically embeds itself to the inserted RON complex. The AMA1–RON2 interaction is necessary to anchor the parasite and form a molecular access point for entry. It is important to note that the MJ functions as a molecular sieve, allowing T. gondii to pass through the host membrane while excluding host plasma membrane proteins. This filtration process allows the parasitophorous vacuole (PV), which will form around the invading parasite, to reflect certain host signaling components to avoid the triggering of immune recognition [33].

Active penetration and PV formation

After the MJ is set up, the parasite goes into the host cell from the MJ direction and pulls the host membrane with it to use for the parasitophorous vacuole (PV). While phagosomes are formed by host cell machinery (e.g. in classical endocytosis), the PV is created by the force exerted by the parasite while still guided by the MJ.

As soon as the vacuole is formed, dense granule proteins (GRAs) (e.g. GRA2, GRA5, GRA7, and GRA15) are secreted into the vacuolar space. These proteins modify the PVM by including parasite-derived lipids and proteins, which covers or masks the PV from autophagy and lysosomal recognition systems in the host cell. The vacuole is considered non-fusogenic-meaning it does not fuse with lysosomes-provide the parasite with the ability to escape degradation [34].

Intracellular survival and replication

Inside the protective PV, T. gondii differentiates into rapidly dividing tachyzoites, the proliferative form of the parasite. The PV supports parasite replication by maintaining a stable environment and by hijacking host cell resources, including nutrients and metabolites. Some dense granule proteins, such as GRA16 and GRA24, are even translocated into the host nucleus, where they modulate host transcriptional programs to suppress pro-inflammatory responses and enhance parasite survival.

This ability to reprogram host signaling from within enables T. gondii to remain largely undetected by the immune system during early infection. In the case of chronic infection, tachyzoites convert into bradyzoites, forming tissue cysts that persist for the lifetime of the host, particularly in immunoprivileged areas like the brain and muscles [35].

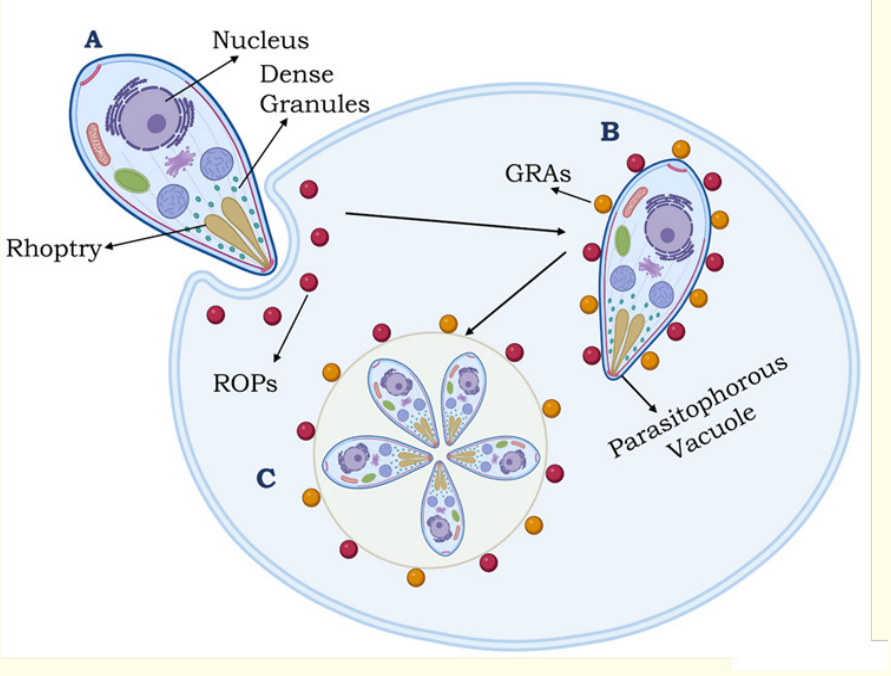

Figure 4: This figure schematically depicts the major steps of the parasitic life cycle, pointing out the most important events occurring during the invasion of the host cell, its survival inside the host cell, and its proliferation. A) Attachment and Entry: The parasite is initially attracted to the host cell by the secretion of proteins released from micronemes and rhoptries. These secretions allow the parasite to invade the host cell membrane and to be encapsulated in a protective parasitophorous vacole, PV. (B) Establishment and Replication: The PV is remodeled in the host cell by ROPs and GRAs, supporting the establishment of the parasite in the PV. Asexual replication of the parasite results in several tachyzoites being produced within the PV. (C) Egress and Further Infection: Once a threshold number of tachyzoites is reached, the host cell bursts, releasing the tachyzoites into the ambient environment for the infection of other cells. This stage might also involve the transformation of tachyzoites to bradyzoites and the formation of tissue cysts, a process that involves chronic infection.

Host-parasite interaction and immune evasion

Toxoplasma gondii has developed intricate mechanisms to manipulate host cell functions and escape immune responses for its survival at acute and chronic stages. These interactions take place at various cellular levels, including mitochondrial hijacking, manipulation of host gene expression, blocking of signaling pathways, and secretion of specialized effector proteins.

Host cell manipulation and parasitophorous vacuole (PV) formation

When the parasite enters the host cell, the parasite T. gondii is encapsulated in a parasitophorous vacuole (PV), which keeps the parasite protected from host lysosomal fusion. T. gondii contains different proteins recognized as rhoptry proteins (ROPs) and dense granule proteins (GRAs). The proteins that are secreted from the rhoptries and dense granules modify the PV membrane to allow the parasite to evade recognition by the host's autophagic machinery. For example, GRA7 and GRA15 take over the host NF-κB signaling, and GRA16 translocates to the host nucleus and changes transcriptional signaling programs to suppress inflammation [36].

Mitochondrial hijacking and host organelle association

T. gondii uses mitochondrial association factor 1b (MAF1b) to recruit host mitochondria to the PV membrane, termed host mitochondrial association (HMA), which modifies host cell metabolism and may dampen detection by innate immune sensors. HMA is strain-dependent and linked to virulence differences between Type I, II, and III lineages [34].

Modulation of host non-coding RNAs

Recent research has demonstrated that T. gondii can manipulate host microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) to regulate immune responses. For example, miR-155 and miR-146a upregulated in infected macrophages and dendritic cells lead to changed cytokine production and suppression of the IFN-γ pathway [see 3]. These changes promote immune evasion and enhance parasite survival during early infection [37].

Chronic infection and immune suppression

Chronic toxoplasmosis is marked by Toxoplasma gondii differentiating into bradyzoites to form tissue cysts, mainly in the brain and muscle tissue. Notably, these cysts evade immune clearance by minimizing inflammation and inhibiting antigen presentation. Cyst reactivation can occur in immunocompromised patients resulting in encephalitis. It has been demonstrated that chronic infection induces downregulation of major histocompatibility complex class I and II (MHC II) molecules on antigen presenting cells, in addition to impairing microglial responses [38].

Interference with host signalling pathways

Toxoplasma gondii employs specialized effector proteins to interfere with key host signaling pathways, thereby subverting immune defenses and promoting its survival. One such effector, ROP18, inactivates immunity-related GTPases (IRGs) in murine cells, which prevents the destruction of the parasitophorous vacuole and allows the parasite to persist intracellularly. Another effector, ROP16, modulates the host immune response by phosphorylating the transcription factors STAT3 and STAT6, leading to the induction of anti-inflammatory pathways that suppress pro-inflammatory cytokine production. Additionally, GRA24 activates the p38 mitogen-activated protein kinase (MAPK) pathway, which alters cytokine profiles in infected cells and contributes to immune modulation. Collectively, these effectors finely tune host signaling networks to create a cellular environment favorable for parasite replication and immune evasion [39].

Immune response to *toxoplasma gondii*

The immune response to *T. gondii* infection is quite complex and is interlinked with innate and adaptive mechanisms while attempting to control the infection. Such an understanding is very necessary while making therapeutic strategies or public health interventions.

Innate immune response

The innate immune response has been regarded as the first-line defense mechanism against infection by *T. gondii*. Thus, the recognition of parasites could be through PRRs, including TLRs, which then would trigger pro-inflammatory cytokines, particularly IL-12, and IFN-γ expressions by macrophages and dendritic cells, so NK cells would further be activated to enhance anti-parasitic activity [47].

Nevertheless, the inbred genetic background of *T. gondii* strain and/or host variants means that results are oftenconsiderably different between studies. While most experiments have been conducted in murine models, and more specifically inbred strains of C57BL/6 mice, these are unfortunately far too simplistic compared to the human immune response. This also means that any findings about species-specific immune regulations, including differences in the cytokine signaling or recognition by PRRs, must be interpreted with caution and taken further to human infections with some reservations [40].

Adaptive immune response

Adaptive immunity would lead, following the innate response, to the control and elimination of *T. gondii*. CD4+ Th1 cells and cytotoxic CD8+ T lymphocytes are considered to be the most important adaptive immune cells in this respect. IFN-γ-producing Th1 cells augment the microbicidal activity of macrophages and facilitate the killing of intracellular parasites. CD8+ T cells kill infected host cells directly and, thus, contribute much to the containment of the infection.

B cells also play a role in the adaptive response by generating *T. gondii*-specific antibodies, which serve to neutralize the parasite and prevent its further spread within the host. A typical feature of this adaptive reaction is the granulomatous response in which the inflammatory cells cluster around the CNS to encapsulate the pathogen.

Thus, although murine models provided insight into diseases caused by acute infections, most of these studies could not represent chronic human toxoplasmosis with its characteristics of immune regulation and parasite persistence. Therefore, studies of chronic infection models and humanized mice or in vitro systems involving human immune cells should be helpful to furnish more pertinent insight into the human immune response to *T. gondii* [41].

CNS pathology in chronic *T. gondii* infection

Chronic infection with Toxoplasma gondii shows a very complex effect on the CNS. From such neurological and behavioral disorders arising out of toxoplasmosis, its persistence in neurons and glial cells of the brain has been cited. Hence, impacts on neuronal functioning, neurogenesis, and overall brain health are discussed along with treatment strategies which may be effective in mitigating such dire consequences.

Neuronal function

The chronic infection of the CNS with T. gondii disrupts normal neuronal function, ultimately leading to cognitive and behavioral abnormalities. Toxoplasma forms the tissue cysts in neural tissues that later provoke a state of chronic inflammation; the latter is characterized by activated microglial and astrocytes, which are the key immune effector cells of the brain. These cytokines consist of proinflammatory IL-1β, IL-6, and TNF-α, which are sustained in their release. Such an inflammatory milieu thus can interfere with important synaptic functions critical to neuronal communication [42].

Synaptic function and neurotransmitter imbalance

Chronic inflammation reduces the number of synapses and alters neurotransmitter levels in a way that normal brain functions are impaired. The most significant neurotransmitters influenced by *T. gondii* are dopamine, glutamate, and serotonin. For example, the infection has been linked to increased production of dopamine, contributing to behavioral changes, neuropsychiatric disorders such as schizophrenia, and bipolar disorder. Altered glutamate signaling may lead to excitotoxicity, in which neurons are over-excited to the extent of injury, leading to further cognitive impairments [43,44].

Neuroinflammation and neuronal death

Neurodegeneration appears to be related to the chronic inflammatory response evoked by *T. gondii* infection, with overactivity of microglial and astrocyte cells potentiated by further production of ROS/NO through relevant enzyme inductions, leading to oxidative stress with consequent neuronal death. Chronic neuroinflammation encourages synaptic dysfunction hence deficits in learning- and memory-related and other cognitive functions [42,44].

Neurogenesis

Toxoplasma gondii has been implicated in influencing the neurogenesis process when neural entities get developed into neurons. Neurogenesis is one of the bases for cognition, mainly at the hippocampus, a very crucial brain area for learning and memory [44].

Disruption of neural progenitor cells

There is a wealth of evidence proving that *T. gondii* infects neural progenitor cells and disrupts their normal course. In such an infection, the normal course of these cells, that give rise to mature neurons, reduces and impairs proliferation. Chronic inflammation, mediated by high cytokines, for example INF-γ and TNF-α, includes inhibition of NPCs cells' proliferation and enhancement of apoptosis to reduce the pool of cells used in neurogenesis. It associates with cognitive impairments, more specifically, in tasks relying on spatial memory and learning [42,43].

Altered cell cycle dynamics

T. gondii, with context dependency, could either arrest the cell cycle of NPCs or drive them toward apoptosis. Such discoordination in the NPC cell cycle will reduce neurogenesis on one end and, through chronic infection, bring structural modification to the brain. Hippocampal atrophy has been linked to the chronic infection of *T. gondii* by colimidating individuals to have cognitive deficits and impairments in memory [45].

Long-term implications

The chronic *T. gondii* infection causes disrupted neurogenesis and altered neuronal function, possibly leading to long-term effects. The alterations, in turn, trigger neuropsychiatric disorders, cognitive decline, and probably increase the risk of neurodegenerative diseases. That is, persistence of *T. gondii* in the CNS continually threatens reactivation, especially in immunosuppressed individuals, which may exacerbate pathological effects [43].

Behavioral and Cognitive Consequences: Behavioral and cognitive shift subsequent to a *T. gondii* infection takes place because of disturbed normal neurogenesis and neuronal functioning. Thus, risk proneness could predict changes in responses towards fright and anxiety, besides deficits in learning and memory in the infected individual. The changes will be a direct consequence of dysregulation in the neurotransmitter systems at the level of the infection of structural changes within the brain.

Epidemiological studies have linked *T. gondii* seropositivity with the risk of neuropsychiatric disorders, such as schizophrenia, bipolar disorder, and personality changes. Such associations would most likely be multi-factorial, with possible involvement of direct parasite effects, immune-mediated damages, and neurotransmitter dysregulation [44,45].

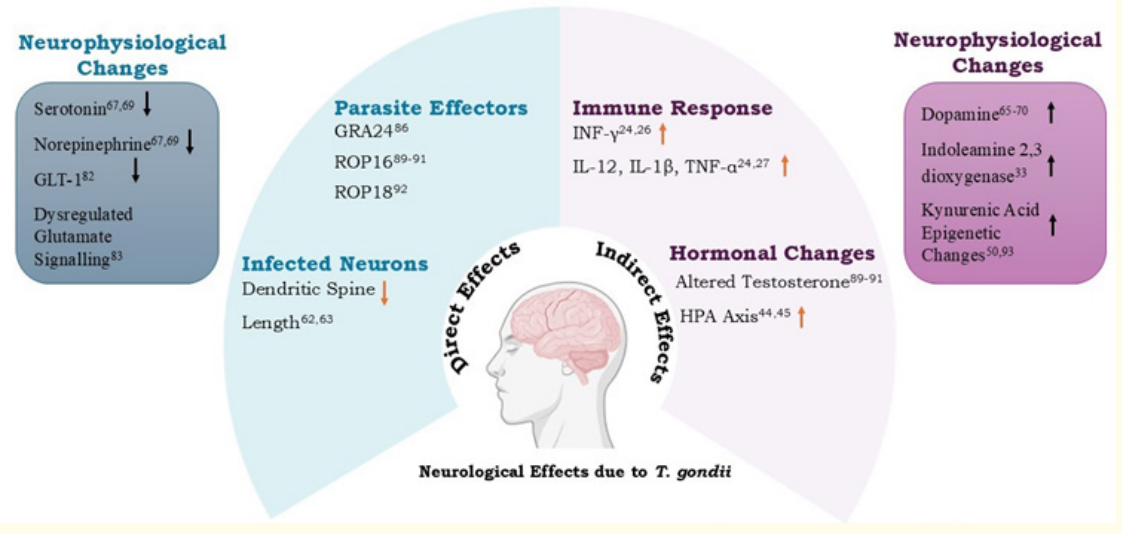

Figure 5: The figure illustrates direct and indirect neurological mechanisms of Toxoplasma gondii, including altered neurotransmitter levels and immune responses.

Diagnostic approaches for toxoplasmosis

Accurate diagnosis of toxoplasmosis is essential for appropriate clinical management, particularly in high-risk populations such as immunocompromised individuals, pregnant women, and neonates. A variety of diagnostic techniques are employed depending on the stage of infection, clinical symptoms, and patient-specific factors. These include serological assays, molecular diagnostics, histopathological evaluation, imaging techniques, and invasive procedures such as amniocentesis and lumbar puncture.

Serological testing

Serological testing continues to serve as the gold standard for initial diagnosis of toxoplasmosis. Enzyme-linked immunosorbent assay (ELISA) and immunosorbent agglutination assay (ISAGA) are the primary testing strategies for the identification of specific Toxoplasma gondii IgM and IgG antibody class. The presence of IgM antibody typically indicates recent or acute infection, while the presence of IgG antibody indicates past exposure or chronic infection. Importantly, IgM antibodies may persist for months, and unless careful consideration is given, this often makes the distinction between acute and latent infection impossible. Because of this, healthcare providers often rely additional testing for IgG avidity, which is a measurement of the binding strength of the IgG antibody. Low avidity IgG indicates recent infection, whereas high avidity IgG indicates that the infection occurred at least months ago. This is particularly relevant for pregnant patients, since the time of maternal infection will predict the risk of congenital transmission [46].

Molecular diagnostics

The ability of the polymerase chain reaction (PCR) assays targeting the B1 gene or the 529-bp repetitive element of T. gondii to direct detect parasite DNA in biological fluids such as blood, cerebrospinal fluid (CSF), amniotic fluid and ocular fluid is highly sensitive and specific. PCR is important in immunocompromised patients, where the host antibody production may be inadequate and in prenatal diagnosis to confirm fetal infection [47].

Histopathology and tissue biopsy

In rare or unclear cases, tissue biopsy may be performed to identify tachyzoites or tissue cysts in affected organs. Histopathological staining techniques, such as hematoxylin and eosin (H and E), or immunohistochemical methods may be used to visualize *T. gondii*. This method is typically reserved for cases involving severe or localized organ involvement (e.g., myocarditis, lymphadenitis, or encephalitis) where non-invasive tests yield inconclusive results [47].

Neuroimaging techniques

For suspected cerebral toxoplasmosis, especially in immunosuppressed patients (e.g., HIV/AIDS), computed tomography (CT) and magnetic resonance imaging (MRI) are critical tools. MRI is preferred due to its superior resolution and sensitivity. Imaging often reveals ring-enhancing lesions, predominantly in the basal ganglia or corticomedullary junction, accompanied by surrounding edema. These findings, in conjunction with clinical presentation and serological/molecular results, guide diagnosis and treatment [45].

Lumbar puncture and CSF analysis

In cases of suspected neurotoxoplasmosis, lumbar puncture may be performed to obtain CSF for diagnostic analysis. PCR can detect T. gondii DNA in CSF, and cytological and biochemical analyses may reveal elevated protein levels and lymphocytic pleocytosis. However, a negative PCR does not rule out the disease, as the parasite load in CSF may be low [47].

Prenatal diagnostics: amniocentesis

In pregnant individuals diagnosed with acute toxoplasmosis, amniocentesis is recommended to assess fetal infection risk, generally performed after 18 weeks of gestation when fetal kidney function ensures the presence of parasite DNA in amniotic fluid. PCR analysis of the fluid can directly detect *T. gondii* DNA. This procedure is often paired with maternal-fetal monitoring and imaging to assess the impact of infection [48].

Prenatal imaging: ultrasound

Ultrasound examination is a non-invasive modality used to identify fetal anomalies associated with congenital toxoplasmosis, including intracranial calcifications, ventriculomegaly, hepatosplenomegaly, and intrauterine growth restriction (IUGR). While ultrasonography cannot confirm infection, it provides important clinical insight and supports the decision for further testing or therapeutic intervention [47,48].

Ocular examination

In cases of ocular toxoplasmosis, particularly in immunocompetent individuals, diagnosis is often clinical and based on characteristic findings such as retinochoroiditis with vitritis, commonly referred to as a "headlight in the fog" appearance. Serology and ocular fluid PCR may be used to support diagnosis in atypical presentations [47,48].

New and emerging diagnostic tools

Recent advances in diagnostic technology include the development of rapid diagnostic tests (RDTs) and biosensors capable of detecting *T. gondii* antigens or antibodies in point-of-care settings. Moreover, proteomics and metabolomics approaches are under investigation to identify novel biomarkers for early detection and prognosis of toxoplasmosis [47,48].

Treatment strategies for *toxoplasma gondii* infection

Treatment of Toxoplasma gondii infection remains a clinical challenge, particularly due to the parasite's complex life cycle and its ability to persist in host tissues in a dormant form. The current standard therapy for acute toxoplasmosis involves a combination of pyrimethamine and sulfadiazine, supplemented with folinic acid (leucovorin) to reduce hematologic toxicity. Pyrimethamine acts by inhibiting dihydrofolate reductase, thereby impairing DNA synthesis, while sulfadiazine inhibits dihydropteroate synthase, both of which are crucial enzymes in the parasite's folate metabolism. This combination is primarily effective against the tachyzoite stage and is widely used in immunocompromised patients with cerebral toxoplasmosis, cases of congenital toxoplasmosis, and other severe symptomatic infections. However, these drugs are associated with several limitations, including gastrointestinal side effects, rash, bone marrow suppression, and the need for prolonged treatment durations ranging from four to six weeks. In patients allergic to sulfa drugs or intolerant to standard regimens, alternative therapies are often employed [49].

Atovaquone, an inhibitor of the mitochondrial electron transport chain, has shown promise in preclinical models against both tachyzoites and bradyzoites. Clindamycin, which inhibits protein synthesis by targeting the 50S ribosomal subunit, is frequently used in combination with pyrimethamine in sulfa-intolerant patients. Other alternatives such as trimethoprim-sulfamethoxazole (TMP-SMX), azithromycin, dapsone, and spiramycin are employed in specific clinical contexts, including prophylaxis and treatment during pregnancy. While these alternatives can reduce toxicity and improve tolerability, their efficacy remains largely restricted to the acute stage of infection, and they share a common limitation with conventional therapies: the inability to eliminate the parasite's latent tissue cysts.

This therapeutic limitation becomes particularly evident during the chronic stages of infection, where T. gondii persists as bradyzoites within intracellular cysts, primarily in the brain and muscle tissues. These cysts are metabolically quiescent and protected by a cyst wall that impairs drug penetration. Moreover, because many anti-parasitic drugs target replicating organisms, the slow-dividing or dormant nature of bradyzoites renders them inherently resistant. Additionally, the location of these cysts in immune-privileged sites, such as the central nervous system, poses a further obstacle for drug delivery. Consequently, current treatment regimens fail to eradicate latent infection, which can later reactivate in immunocompromised individuals and cause life-threatening conditions such as toxoplasmic encephalitis. The persistence of bradyzoites despite therapy highlights a critical gap in the management of toxoplasmosis and underscores the urgent need for the development of novel therapeutic agents capable of targeting chronic infection stages [50].

Emerging treatment strategies

Emerging treatment strategies for *Toxoplasma gondii* aim to overcome the limitations of conventional therapies, particularly their inability to effectively target the bradyzoite cyst stage and issues related to drug toxicity and resistance. These strategies focus on novel molecular targets unique to the parasite, host-pathogen interactions, and advanced drug delivery technologies [49].

Endochin-like quinolones (ELQs) represent a promising class of compounds that inhibit the mitochondrial cytochrome bc1 com-

plex, an essential component of the parasite's electron transport chain responsible for energy production. By blocking this complex, ELQs disrupt the parasite's mitochondrial respiration, leading to parasite death. Notably, ELQs exhibit activity not only against the rapidly replicating tachyzoites but also against the dormant bradyzoite cysts, which are refractory to most current treatments. This dual-stage efficacy is critical for achieving radical cure and preventing disease reactivation, especially in immunocompromised individuals. Preclinical animal studies have demonstrated ELQs' potent anti-parasitic activity with favorable pharmacokinetic properties, marking them as strong candidates for further development [49,51].

Calcium-dependent protein kinase inhibitors (CDPK inhibitors) target CDPK1, a kinase enzyme uniquely expressed by *T. gondii* and related apicomplexan parasites. CDPK1 regulates key processes such as parasite motility, host cell invasion, and egress, making it an attractive drug target. Inhibitors designed against CDPK1 interfere with these essential functions, effectively halting parasite proliferation. Since human cells lack CDPK1 homologs, these inhibitors offer the potential for selective parasite toxicity with minimal off-target effects. Several small molecule inhibitors, including bumped kinase inhibitors (BKIs), have shown encouraging results in in vitro and animal model studies, reducing parasite burden and disease severity [51].

Host immune modulation is another promising avenue. Since *T. gondii* subverts host immunity to establish infection, therapeutics that boost host immune responses could enhance parasite clearance. Agents that stimulate the production of interferon-gamma (IFN-γ), a cytokine critical for activating macrophages and controlling parasite replication, have demonstrated efficacy in preclinical models. Additionally, induction of autophagy pathways in host cells may facilitate the elimination of intracellular parasites. These immunomodulatory approaches could complement direct antiparasitic drugs, especially for chronic infections [50,52].

Nanoparticle-based drug delivery systems are being explored to improve drug bioavailability, stability, and targeted delivery. Many anti-toxoplasma drugs have limited ability to cross the blood-brain barrier (BBB), restricting their efficacy in treating cerebral toxoplasmosis. Encapsulation of drugs such as pyrimethamine or atovaquone within nanoparticles can enhance penetration into the central nervous system, improve pharmacokinetics, and reduce systemic toxicity. Moreover, nanoparticles can be engineered for controlled release and targeted delivery to infected tissues, potentially improving treatment outcomes and patient compliance [53].

New and experimental treatments

New and experimental treatment options for *T. gondii* infection are expanding rapidly with advances in molecular biology, pharmacology, and immunology. These approaches explore novel chemical entities, genetic tools, and immunotherapies to provide more effective and safer interventions.

Bumped kinase inhibitors (BKIs) are a subclass of CDPK1 inhibitors designed to achieve high specificity and potency against *T. gondii*. BKIs exploit unique structural features of the parasite's kinase ATP-binding pocket that differ from human kinases. These molecules disrupt parasite motility, invasion, and egress, key steps in the infectious cycle. Animal studies have shown that BKIs reduce parasite load in acute and chronic models of infection with low toxicity. Some BKIs are advancing towards clinical trials, underscoring their therapeutic potential [51].

Vaccine development represents a critical area of research aimed at preventing toxoplasmosis, especially congenital transmission and disease in immunocompromised individuals. Experimental vaccines include recombinant protein subunits, DNA vaccines, and live attenuated strains engineered to induce robust cellular immunity. Vaccines target key parasite antigens such as surface proteins and secretory effectors involved in host invasion and immune evasion. Although no vaccine is yet approved for human use, several candidates have demonstrated promising immunogenicity and protective efficacy in animal models [52].

Drug repurposing offers a pragmatic approach to accelerate treatment availability by identifying existing FDA-approved drugs with anti-toxoplasma activity. For example, artemisinin derivatives, widely used as antimalarials, exhibit antiparasitic effects against *T. gondii* in vitro and in vivo, possibly through generation of reactive oxygen species and interference with parasite metabolism. Similarly, certain antifungal agents like clotrimazole and its derivatives inhibit parasite growth by disrupting membrane integrity or calci-

um homeostasis. Drug repurposing reduces the timeline and costs associated with drug development since safety profiles are already established [49,50].

CRISPR-Cas9 gene editing technologies have revolutionized *T. gondii* research by enabling precise genetic manipulation of the parasite. This tool allows identification of essential genes and drug targets by creating knockout or mutant parasite strains. Beyond research, CRISPR-based approaches hold potential for developing genetically attenuated parasites that could serve as live vaccines. Although still in early experimental stages, gene editing is accelerating discovery of novel therapeutic targets and informing rational drug design [51].

Herbal and alternative treatments

Besides the current symptomatic treatments, a place must be given to alternative therapeutic approaches that include those employing herbal plants. The present section is developed as an in-depth analysis regarding herbal and other unconventional treatments that have captured interest because of their potential added benefits in complementing or further improving traditional therapies. Because this section covers alternative strategies immediately after the conventional methods, it provides a natural progression from well-established practices to treatment modalities that are more innovative or less widely accepted. The organization does more-it points to an emerging interest in herbal treatments and sets the stage for the exploration of emerging and experimental therapies [55].

Herbal Plant	Common Name	Plant Part Used	Traditional Use and Reported Effects
Artemisia annua	Sweet Wormwood	Leaves	Used in traditional Chinese medicine; has anti-parasitic and anti-inflammatory properties (Sprowls., *et al*., 2019)
Azadirachta indica	Neem	Leaves, Bark	Used in Ayurvedic medicine; exhibits anti-parasitic and immunomodulatory effects · (Mantovani., *et al*., 2009)
Allium sativum	Garlic	Bulb	Widely used as a culinary herb; has antimicrobial, antifungal, and antiparasitic properties (Kurniawan., *et al*., 2013)
Berberis vulgaris	Barberry	Bark, Roots	Used in traditional medicine; contains berberine, which has antimicrobial and anti-parasitic properties (Vallochi., *et al*., 2015)
Curcuma longa	Turmeric	Rhizome	Widely used as a spice; contains curcumin, which has antimicrobial and immunomodulatory properties (Nayeri., *et al*., 2021)
Echinacea purpurea	Purple Coneflower	Aerial Parts	Utilized in herbal remedies; known for its immune-enhancing and anti-inflammatory effects (Côté., *et al*., 2013)
Uncaria tomentosa	Cat's Claw	Bark, Roots	Used in traditional medicine; has immunomodulatory and anti-inflammatory properties (Dubey., *et al*., 2005)

Table 1: Table presenting a list of available herbal plant, their common uses, and their mechanisms of action against *T. gondii* infection.

Conclusion

Toxoplasma gondii remains one of the most widespread and medically significant parasitic infections worldwide, particularly due to its ability to establish lifelong latent infections and reactivate under conditions of immune suppression. As this review highlights, the parasite's success lies in its remarkable arsenal of molecular tools that enable host cell invasion, immune evasion, and persistence within a wide range of tissues, especially in immune-privileged sites like the brain and eye.

Recent advances in molecular parasitology have significantly deepened our understanding of the mechanisms governing T. gondii pathogenesis. Key discoveries include the roles of rhoptry and dense granule proteins in modulating host signaling pathways, the hijacking of host organelles such as mitochondria, and the manipulation of host non-coding RNAs to suppress immune responses. These strategies collectively enable the parasite to evade immune clearance, differentiate into bradyzoite cysts, and maintain a latent state.

Despite this growing body of knowledge, major gaps remain-particularly in understanding how chronic infection is maintained at the molecular level, how cyst reactivation is triggered, and how best to target bradyzoites pharmacologically. Current therapies are limited to the acute stage and often have significant toxicity. However, promising experimental treatments such as bumped kinase inhibitors, host-directed therapies, and nanoparticle-based drug delivery systems are under active investigation. Additionally, the application of CRISPR-Cas9 technologies has opened new avenues in functional genomics, accelerating the identification of essential genes and novel drug targets.

Moving forward, integrating omics technologies, high-throughput screening, and immunotherapeutic approaches will be critical in designing more effective and stage-specific interventions. A deeper understanding of host–parasite interactions will not only improve treatment strategies but also inform vaccine development and public health policies aimed at reducing the burden of toxoplasmosis.

By consolidating recent insights into T. gondii biology, this review underscores the importance of mechanistic research in shaping future therapeutic and preventive measures against this persistent and adaptable pathogen.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All the cited authors have given their approval for the work to be published; they have all made substantial, direct, and intellectual contributions.

Bibliography

1. Kochanowsky Joshua A and Anita A Koshy. "Toxoplasma gondii". *Current Biology : CB* 28.14 (2018): R770-R771.
2. Attias Márcia., *et al.* "The life-cycle of Toxoplasma gondii reviewed using animations". *Parasites and Vectors* 13.1 (2020): 588.
3. Hunter Christopher A and L David Sibley. "Modulation of innate immunity by Toxoplasma gondii virulence effectors". *Nature Reviews. Microbiology* 10.11 (2012): 766-778.
4. Medina Lisvaneth., *et al.* "*Trypanosoma cruzi* and *Toxoplasma gondii* Induce a Differential MicroRNA Profile in Human Placental Explants". *Frontiers in Immunology* 11 (2020): 595250.
5. Pan Ming., *et al.* "The determinants regulating *Toxoplasma gondii* bradyzoite development". *Frontiers in Microbiology* 13 (2022): 1027073.
6. Dunay Ildiko Rita., *et al.* "Treatment of Toxoplasmosis: Historical Perspective, Animal Models, and Current Clinical Practice". *Clinical Microbiology Reviews* 31.4 (2018): e00057-17.
7. Elsheikha Hany M., *et al.* "Epidemiology, Pathophysiology, Diagnosis, and Management of Cerebral Toxoplasmosis". *Clinical Microbiology Reviews* 34.1 (2022): e00115-119.
8. Elmore SA., *et al.* "Toxoplasma gondii: Epidemiology and Control". *Veterinary Parasitology* 170.2-4 (2010): 145-153.
9. Furtado João M., *et al.* "Toxoplasmosis: A Global Threat". *Current Ophthalmology Reports* 4.1 (2016): 67-74.
10. Petersen Esben., *et al.* "What Do We Know About Risk Factors for Infection in Humans with Toxoplasma gondii and How Can We Prevent Infections?" *Zoonoses and Public Health* 57.1 (2010): 8-17.
11. Flegr Jaroslav., *et al.* "Toxoplasmosis: A Global Threat and Its Possible Links to Mental Disorders and Behavioral Changes". *Folia Parasitologica* 61.6 (2014).
12. Saadatnia Gilda and Davood Golkar. "A Review on Human Toxoplasmosis". *Scandinavian Journal of Infectious Diseases* 44.11 (2012): 805-814.

13. Attias Márcia., *et al.* "The life-cycle of Toxoplasma gondii reviewed using animations". *Parasites and Vectors* 13.1 (2020): 588.

14. Tenter Astrid M., *et al.* "Toxoplasma gondii: from animals to humans". *International journal for Parasitology* 30.12-13 (2000): 1217-1258.

15. Dubey JP. "Advances in the life cycle of Toxoplasma gondii". *International Journal for Parasitology* 28.7 (1998): 1019-1024.

16. Gissot Mathieu. "Toxoplasma gondii: Asexual Cycle in the Intermediate Host". *Lifecycles of Pathogenic Protists in Humans*. Cham: Springer International Publishing (2022): 391-417.

17. Jeffers Victoria., *et al.* "A latent ability to persist: differentiation in Toxoplasma gondii". *Cellular and Molecular Life Sciences : CMLS* 75.13 (2018): 2355-2373.

18. Sullivan William J and Victoria Jeffers. "Mechanisms of Toxoplasma gondii persistence and latency". *FEMS Microbiology Reviews* 36.3 (2012): 717-33.

19. Nayeri Tooran., *et al.* "Effective factors in the pathogenesis of *Toxoplasmagondii*". *Heliyon* 10.10 (2024): e31558.

20. Dubey JP., *et al.* "Structures of Toxoplasma gondii tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts". *Clinical Microbiology Reviews* 11.2 (1998): 267-299.

21. McFadden Geoffrey Ian and Ellen Yeh. "The apicoplast: now you see it, now you don't". *International Journal for Parasitology* 47.2-3 (2017): 137-144.

22. Kim Kami. "A Bradyzoite is a Bradyzoite is a Bradyzoite?". *Trends in Parasitology* 31.12 (2015): 610-612.

23. Cerutti Aude., *et al.* "The Bradyzoite: A Key Developmental Stage for the Persistence and Pathogenesis of Toxoplasmosis". *Pathogens (Basel, Switzerland)* 9.3 (2020): 234.

24. Dubey JP. "Unexpected oocyst shedding by cats fed Toxoplasma gondii tachyzoites: in vivo stage conversion and strain variation". *Veterinary Parasitology* 133.4 (2005): 289-298.

25. Kidaka Taishi., *et al.* "TSS-seq of Toxoplasma gondii sporozoites revealed a novel motif in stage-specific promoters". *Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases* 98 (2022): 105213.

26. Poukchanski Anna., *et al.* "Toxoplasma gondii sporozoites invade host cells using two novel paralogues of RON2 and AMA1". *PloS One* 8.8 (2013): e70637.

27. Morrissette Naomi S and L David Sibley. "Cytoskeleton of apicomplexan parasites". *Microbiology and Molecular Biology Reviews: MMBR* 66.1 (2002): 21-38.

28. Pasquarelli Rebecca R., *et al.* "Characterization and functional analysis of *Toxoplasma* Golgi-associated proteins identified by proximity labeling". *mBio* 15.11 (2024): e0238024.

29. Dubremetz Jean François and Maryse Lebrun. "Virulence factors of Toxoplasma gondii". *Microbes and Infection* 14.15 (2012): 1403-1410.

30. Arisue Nobuko and Tetsuo Hashimoto. "Phylogeny and evolution of apicoplasts and apicomplexan parasites". *Parasitology International* 64.3 (2015): 254-259.

31. Whitelaw Jamie A., *et al.* "Surface attachment, promoted by the actomyosin system of Toxoplasma gondii is important for efficient gliding motility and invasion". *BMC Biology* 15.1 (2017): 1.

32. Katris Nicholas J., *et al.* "The apical complex provides a regulated gateway for secretion of invasion factors in Toxoplasma". *PLoS Pathogens* 10.4 (2014): e1004074.

33. Shen Bang and L David Sibley. "The moving junction, a key portal to host cell invasion by apicomplexan parasites". *Current Opinion in Microbiology* 15.4 (2012): 449-455.

34. Goldberg Daniel E and Joshua Zimmerberg. "Hardly Vacuous: The Parasitophorous Vacuolar Membrane of Malaria Parasites". *Trends in Parasitology* 36.2 (2020): 138-146.

35. Sanchez Syrian G and Sébastien Besteiro. "The pathogenicity and virulence of *Toxoplasma gondii*". *Virulence* 12.1 (2021): 3095-3114.

36. Portes Juliana., *et al.* "*Toxoplasma gondii* Mechanisms of Entry Into Host Cells". *Frontiers in Cellular and Infection Microbiology* 10 (2020): 294.

37. Menard Kayla L., *et al.* "Impact of *Toxoplasma gondii* Infection on Host Non-coding RNA Responses". *Frontiers in Cellular and Infection Microbiology* 9 (2019): 132.

38. Zhao Xiao-Yu and Sarah E. Ewald. "The molecular biology and immune control of chronic Toxoplasma gondii infection". *The Journal of Clinical Investigation* 130.7 (2020): 3370-3380.v

39. Hakimi Mohamed-Ali., *et al.* "*Toxoplasma* Effectors Targeting Host Signaling and Transcription". *Clinical Microbiology Reviews* 30.3 (2017): 615-645.

40. Montoya Jose G and Jack S Remington. "Toxoplasma gondii". In *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, edited by John E. Bennett., *et al*., 9th edition., Elsevier (2020): 3503-3520.

41. Robert-Gangneux Florence., *et al.* "New Molecular Diagnostic Tools for the Diagnosis of Maternal and Congenital Toxoplasmosis: Perspectives for 2030". *Microorganisms* 10.9 (2022): 1737.

42. Pomares Christelle and Pierre-Yves Levy. "Toxoplasmosis and Transplantation". *Transplant Infectious Disease* 22.3 (2020): e13213.

43. McAuley James B. "Congenital Toxoplasmosis". *Journal of the Pediatric Infectious Diseases Society* 3.1 (2014): S30-S35.

44. Halonen Sandra K and John P Weiss. "Toxoplasmosis". *Handbook of Clinical Neurology* 114 (2013): 125-145.

45. Dunay Ian R., *et al.* "Toxoplasma gondii and Neurodegeneration". *Trends in Parasitology* 34.4 (2018): 261-272.

46. Villard O., *et al.* "Serological diagnosis of Toxoplasma gondii infection: Recommendations from the French National Reference Center for Toxoplasmosis". *Diagnostic Microbiology and Infectious Disease* 84.1 (2016): 22-33.

47. Switaj K., *et al.* "Recent trends in molecular diagnostics for Toxoplasma gondii infections". *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases* 11.3 (2005): 170-176.

48. Hill D and J P Dubey. "Toxoplasma gondii: transmission, diagnosis and prevention". *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases* 8.10 (2002): 634-640.

49. Müller Joachim and Andrew Hemphill. "*Toxoplasma gondii* infection: novel emerging therapeutic targets". *Expert Opinion on Therapeutic Targets* 27.4-5 (2023): 293-304.

50. Müller Joachim and Andrew Hemphill. "*Toxoplasma gondii* infection: novel emerging therapeutic targets". *Expert Opinion on Therapeutic Targets* 27.4-5 (2023): 293-304.

51. Anghel N., *et al.* "Endochin-like quinolones (ELQs) and bumped kinase inhibitors (BKIs): Synergistic and additive effects of combined treatments against *Neospora caninum* infection *in vitro* and *in vivo*". *International Journal for Parasitology: Drugs and Drug Resistance* 17 (2021): 92-106.

52. Mévélec Marie-Noëlle., *et al.* "Key Limitations and New Insights Into the *Toxoplasma gondii* Parasite Stage Switching for Future Vaccine Development in Human, Livestock, and Cats". *Frontiers in Cellular and Infection Microbiology* 10 (2020): 607198.

53. Assolini João Paulo., *et al.* "Nanomedicine advances in toxoplasmosis: diagnostic, treatment, and vaccine applications". *Parasitology Research* 116 (2017): 1603-1615.

54. Doggett J Stone., *et al.* "Bumped kinase inhibitor 1294 treats established Toxoplasma gondii infection". *Antimicrobial Agents and Chemotherapy* 58.6 (2014): 3547-3549.

55. Al Nasr., *et al.* "Toxoplasmosis and anti-Toxoplasma effects of medicinal plant extracts-A mini-review". *Asian Pacific Journal of Tropical Medicine* 9.8 (2016): 730-734.